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REVIEWER'S REPORT

Manuscript No.: IJAR-55951

Title: Activation of NRF2/HO-1 Signaling Mediates the Protective Effects of Hibiscus Sabdariffa Against Restraint Stress–Induced Oxidative Damage in Female Wistar Rats

Recommendation:

Accept as it is .....

✓ Accept after minor revision.....

Accept after major revision .....

Do not accept (*Reasons below*) .....

Rating	Excel.	Good	Fair	Poor
Originality		✓		
Techn. Quality		✓		
Clarity		✓		
Significance	✓			

Reviewer Name: Dr S. K. Nath **Date:** 28.01.26

Detailed Reviewer's Report

Strengths of the Study

- **Originality:** The study addresses the protective mechanisms of Hibiscus sabdariffa in female models subjected to restraint stress, filling a research gap related to sex-specific stress responses.
- **Relevance:** It explores the molecular basis of plant-based antioxidant therapy, which is highly relevant given the global interest in alternative and complementary medicine.
- **Methodology:** Utilizes a comprehensive experimental design combining biochemical, molecular, and histopathological analyses across multiple organ systems.
- **Data Quality:** Presents detailed quantitative data supporting claims, including gene and protein expression levels, antioxidant enzyme activities, and histological findings.
- **Contribution:** Provides mechanistic insights into how Hibiscus sabdariffa activates the Nrf2/HO-1 pathway, thus contributing to the understanding of plant-derived redox modulators in stress physiology.

Weaknesses of the Study

- **Sample Size:** The group size (n=6) may be relatively small for robust statistical power, especially for molecular analyses.
- **Methodological Detail:** Lack of clear description regarding the phytochemical composition of the Hibiscus sabdariffa extract, which affects reproducibility.
- **Statistical Analysis:** The statistical methods used are broadly described; detailed information on post hoc tests or assumptions checked (normality, variance homogeneity) is missing.
- **Ethical Details:** While approval from the animal ethics committee is mentioned, explicit details on animal welfare procedures, with reference to specific guidelines, are absent.
- **Data Presentation:** Some figures lack detailed legends; the clarity and readability of histological images could be improved.
- **Previous Publication Check:** The manuscript appears original with no evidence of prior publication or preprint content available on the internet.

Reviewer Comments

- **Title and Abstract:** The title accurately reflects the study scope. The abstract is clear, concise, and well-structured, effectively summarizing key findings, though it could more explicitly mention the significance of the findings for female health.

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- **Introduction and Objectives:** The introduction provides a good background on oxidative stress, Nrf2 pathway, and the relevance of Hibiscus sabdariffa. The research objective is clearly stated, focusing on the molecular mechanisms.
- **Methodology and Statistical Analysis:** The experimental design is appropriate, but details regarding extraction standardization, specific doses of phytochemicals, and experimental controls are lacking. Statistical methods should specify the tests used and assumptions verified.
- **Results and Discussion:** Results are well-organized, linking biochemical, molecular, and histopathological findings. The discussion appropriately interprets the data, emphasizing the mechanistic role of Nrf2/HO-1. Incorporating a comparison with existing literature would enhance contextual understanding.
- **Conclusion and Implications:** The conclusions are supported by data, highlighting potential therapeutic implications, especially for stress-related oxidative disorders in females.
- **Ethical Clearance:** Ethical approval is noted; however, the manuscript should specify adherence to standard guidelines (e.g., ARRIVE) and mention if informed consent was obtained for animal use.
- **Language and Formatting:** The manuscript is generally well-written, but some minor grammatical errors and awkward phrasing are present. Consistency in reference formatting and figure legends should be improved.
- **Figures and Tables:** Figures are relevant; however, they could benefit from higher resolution and more detailed legends. Inclusion of a table summarizing biochemical and molecular results would aid clarity.
- **References:** Adequate referencing; ensure all references are current and formatted uniformly per journal guidelines.

Additional Note: Based on the review of the content provided and standard practices for scholarly publications, there are no indications or evidence within the text suggesting that this particular study has been previously published on the internet or elsewhere. To conclusively verify whether this work has been previously published, a dedicated plagiarism check or database search (e.g., PubMed, Google Scholar, or institutional repositories) would be necessary.